THE THYROID AXIS AND BRAIN 5'-MONODEIODINATION OF THYROXINE IN THE BURNED RAT MODEL OF NONTHYROIDAL ILLNESS

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ABSTRACT

Rats with a cutaneous burn exhibited a burn-size-related depression of serum thyroid hormones 2 weeks after injury. Thyroidectomized rats had a marked rise in brain in vitro T4 deiodination to T3 and in serum TSH concentration compared to controls. These normal responses to depressed thyroid hormones were not seen in burned rats. The depression of serum thyronines and thyrotrophin suggests that the burned rat may be a model for human burn injury and other nonthyroidal illness. In this model it appears that the brain and pituitary do not respond as in thyroidectomy, suggesting lack of true hypothyroidism in burn injury.

INTRODUCTION

Shirani et al. (1985) have reported depressed total and free concentrations of thyroxine (T4) and triiodothyronine (T3) in burned rats and suggested the burned rat as a model for human nonthyroidal illness (NTI). It is not yet known whether burn injury and other NTI represent conditions of hypothyroidism. We now assess whether serum thyrotrophin (TSH) and brain T4 to T3 deiodinating capacity respond as expected for hypothyroidism in the burn model.

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In this research the investigators adhered to the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and to the Guide for the Care and Use of Laboratory Animals, NIH publication 80-23 (rev.).

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MATERIALS AND METHODS

Adult 350g male rats adapted to a light/dark cycle of 14/10 h (lights on 0600 h) were anesthetized and given standard full-thickness scalds of 20, 40, or 60% of body surface area. Sham-burned rats were anesthetized and exposed to water of room temperature. Other rats were thyroidectomized under anesthesia and maintained with 0.9% CaCl2 in the drinking water. Rats were maintained on laboratory chow and water ad libitum. Groups of 5 rats with each procedure were sacrificed by guillotine at 0800 h on the post-procedure days according to Fig. 1. Control rats without a procedure were sacrificed in groups of 5 at times corresponding to days 8, 12 and 15.

For most groups, the brain (olfactory lobes, midbrain, pons and cerebellum eliminated by razor section) were sagittally hemisected on ice and the left telencephalon and diencephalon were homogenized in two volumes of ice cold 0.1 M pH 7.4 phosphate buffer with 25 mM dithiothreitol. Aliquots of 0.175 ml were incubated for 2 h at 37°C with T4 (1 ug/ml) in a final volume of 0.25 ml, and control aliquots received the T4 only at the end of the incubation. The reaction was stopped by extracting the mixture with 10 volumes of cold ethanol and centrifugation at 2000 G for 30 min at 4°C. Duplicate 0.5 ml supernatant aliquots were evaporated and suspended in assay buffer for determination of T3. These procedures closely resemble published determinations of 5'-deiodination of T4 to T3 in the presence of excess T4 by radioimmunoassay (RIA) of the generated T3 (Chopra, 1977; Chopra, 1978; El-Zaheri et al., 1980; Chopra et al., 1980; Gavin and Moeller, 1983; Chopra et al., 1984; Chopra et al., 1985). The difference in T3 values between conditions of T4 added before and after incubation provided correction for T3 not generated from the added T4 and/or other nonspecific influences and was used to calculate the deiodination index (DI) as ng T3 generated per gram of original tissue.

Serum T4, T3, T3 uptake (T3U, radioassay) and reverse T3 (rT3) were determined with commercial RIA kits (Vaughan et al., 1985a). The free T4 index (FT4I) was the product of the T4 and T3U. In a separate experiment with 27 burned (60% of body surface), 22 sham-burned, 5 thyroidectomized and 11 control rats, only serum thyrotrophin (TSH) was determined by RIA using reagents (including RP-1 standard) from NIADDK, USA. Student t tests were used to

compare two means in relevant comparisons.

RESULTS

Thyroidectomy resulted in depression of serum thyronines and elevation of brain DI and serum TSH. Figure 1 shows that some burn groups had depression of serum T4, FT4I, T3, and rT3 into the range exhibited by thyroidectomized groups. On combined postburn days 14 and 15, each of these hormonal variables correlated negatively with burn size (each $p < 0.001$, not shown). The burned rats failed to show elevation of brain DI. Serum TSH was slightly but significantly depressed in comparison with sham burn.

DISCUSSION

Reduced serum T4, FT4I, and T3 is associated with depressed dialyzable free concentrations of T4 and T3 on days 8 and 14 after a burn of 60% of skin surface in rats (Shirani et al., 1985). These findings often occur in humans with large burns (Vaughan et al., 1985b) or other critical NTI (see Chopra et al., 1983, and Vaughan et al., 1985b). As in burned humans, the depression of these hormones in the presently studied rats was also burn-size related. There is a general lack of metabolic evidence for hypothyroidism at the end-organ level in humans with burns and other NTI, also in whom serum TSH concentration is usually not elevated (Chopra et al., 1983; Wartofsky and Burman, 1982) or is depressed (Becker et al., 1982; Wehman et al., 1985). In rats, non-elevated or reduced serum TSH has been reported in various nonburn models of NTI, despite depressed serum T4 and T3 (Tibaldi and Surks, 1985). Elevated O2 consumption has been reported in burned humans (Vaughan et al., 1985a) and rats (Herndon et al., 1978). But because of the low thyroid hormones and the deficit in mental status in patients with large burns (Vaughan et al., 1985b), the question of hypothyroidism, particularly at the level of the brain, lingers. We utilized the burn rat model to address this issue.

In the rat, a rise in brain 5'-deiodinase activity and local T4 to T3 conversion is the normal response to a primary depression of serum thyroid hormones (Leonard et al., 1981; Larsen et al., 1981), as is a rise in serum TSH. Failure of these responses in the burn rat model supports the argument against physiologic hypothyroidism in burn injury. Something about the presence of burn injury,

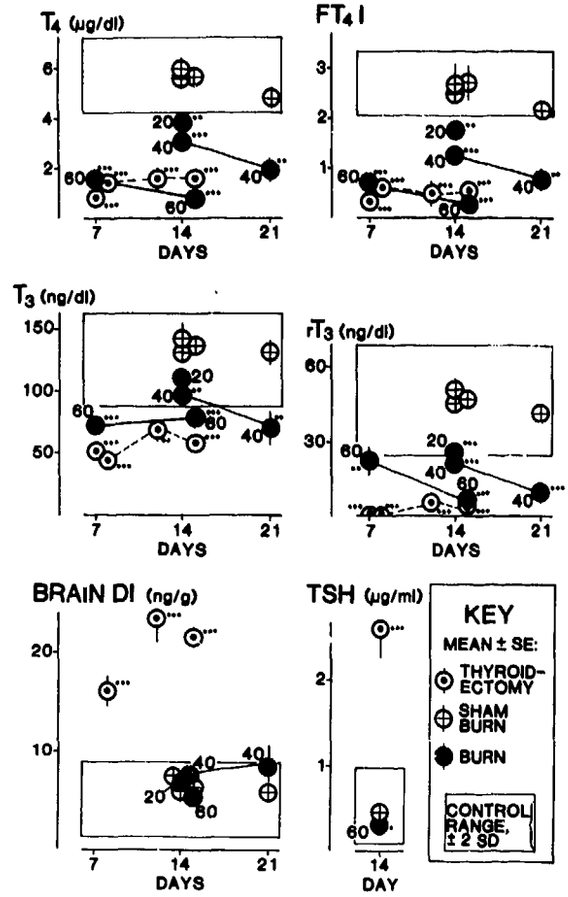

Fig. 1. Thyroid axis hormones and brain T4-to-T3 deiodination index (DI) at the indicated time (DAYS) after burn or thyroidectomy in rats. Numbers near the symbols indicate the burn size as percent of body surface. *p < 0.05, **p < 0.01, ***p < 0.001, burn vs respective sham, or thyroidectomy vs respective controls (except in the upper 4 panels in which the burn group at day 7 without shams is compared to controls). For graphic purposes, the control data were pooled and are used to provide an indication of the normal range (line rectangles, hormone mean ± 2 SD).

probably in common with other NTI, dramatically prevents internal perception of the depressed thyroid hormone levels as low with respect to the brain and pituitary. Reduced TSH levels may contribute to the depression of circulating T4 and T3. It has not yet been determined how much of the thyroid axis response to burns and other NTI in humans and rats is a result of possible relative caloric deprivation (anorexia or food intolerance), but the known hypermetabolism (rather than decreased O2 consumption) in burn injury and febrile NTI is opposite to the finding in starvation and suggests that other signals play a role in the overall metabolic response to injury and illness (Vaughan et al., 1985a).

The reduced serum rT3 in the burned rat appears different from rT3 concentrations at similar post-injury-times in humans with burns (Becker et al., 1982; Vaughan et al., 1985b) and other NTI (Chopra et al., 1983; Wartofsky and Burman, 1982), in whom rT3 concentrations usually are normal or elevated. The mechanism underlying this depression of rT3 is not yet known but appears not to result from crossreactivity of the rT3 assay with T4, since the same rT3 assay was used in studies of burned humans. Further, addition of T4 to a pool of serum from rats with a 60% burn resulting in a T4 concentration equal to the control mean elevated the apparent rT3 concentration from 1.2 only to 6.0 ng/dl instead of to the mean control level of 45 ng/dl. The skin of the rat normally contains much more T4 to rT3 converting activity and also a much higher rT3 concentration than any of several other tissues examined (Huang et al., 1985), indicating that in this species the skin is the major site of rT3 enzymatic formation and storage. Thus, destruction of the skin by a burn in the rat might inhibit rT3 production in a burn-size-related fashion. It is possible to speculate that the different serum rT3 response to burns in humans might result from relatively greater dependence of rT3 production on sites other than skin in humans. However, in burned humans, unlike the dramatic and burn-size related falls of serum T4 and T3, the elevation of rT3 was less consistent and not demonstrably burn-size related (Vaughan et al., 1985b). Thus, a likely complex interaction of responses perhaps in both production and degradation of rT3, might include reduced production in skin possibly offset by responses at other sites in burned humans.

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